entrapment efficiency and spontaneous release of anti-cancer drug doxorubicin is studied by using absorption spectroscopy and fluorescence de-quenching method. These physical measurements are performed on liposomes with varying PLFE molar ratios at different temperatures. The obtained results may help to optimize and design liposomal drugs with greater stability and higher therapeutic efficacy. (supported by NSF DMR1105277)

1272-Pos Board B164
PLFE Lipids Stabilize Liposomal CA4P
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Liposomal encapsulation using bipolar tetraether lipids such as the polar lipid fraction E (PLFE) isolated from the archaeon Sulfolobus acidocaldarius offer many advantages over conventional phospholipid mediated or free drug delivery. PLFE provide increased stability to lipid vesicles. Liposome mediated drug delivery can reduce the off target effects caused by anti-cancer drugs. Therefore, we hypothesize that PLFE archaeosomes, offer the anti-cancer drug combretastatin A4 disodium phosphate (CA4P) a higher therapeutic efficacy by increasing the drug's stability, circulation time, and targeting. In this study, the fluorescent properties of CA4P have been utilized for drug leakage assays with different compositions of PLFE/POPC in unilamellar vesicles of varying sizes. We have found that PLFE lipids stabilize liposomes and decrease rate of CA4P leakage. We have also shown that this effect is manifested in the cytotoxicity assay against human MCF-7 breast cancer cells. (supported by NSF DMR1105277)

1273-Pos Board B165
Hydrodynamic Co-Localization of Molecules in Supported Lipid Bilayers Detected by Secondary Ion Mass Spectrometry
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Here we report on the use of secondary ion mass spectrometry (SIMS) to study the hydrodynamic co-localization of membrane components in supported lipid bilayers formed by the fusion of multi-component giant unilamellar vesicles to oxidized silicon substrates. In these experiments, hydrodynamic drag forces arising from flow above the supported lipid bilayer (SLB) results in the directed motion of molecules protruding from the SLB. In this particular case, protrusion of the cholera toxin B into the aqueous layer serves as a handle for the directed motion of its natural ligand, ganglioside GM1, and any other molecule (i.e. cholesterol) strongly associated with it. Orthogonal isotopic labeling or fluorination of every lipid bilayer component allowed generation of molecule-specific images, using a nanoSIMS, that map the lateral redistribution of molecules in a lipid bilayer as a result of hydrodynamic flow. Furthermore, simultaneous detection of up to seven different ion species, including secondary electrons, allowed generation of ion ratio images whose signal intensity values could be correlated to composition through the use of calibration curves from standard samples.

1274-Pos Board B166
Interaction of 1,4-Naphtoquinone with Cell Membranes Models Studied with Tensiometry and Vibrational Spectroscopy
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Antineoplastic drugs are natural or synthetic compounds that act against the development of cancer cells, whose chemical interactions with cell membranes have a mechanism of action not sufficiently known so far. For this reason, it is imperative the understanding at the molecular level of drug-cell interactions, and using models for cell membranes is a suitable strategy for that. In this study, we employed Langmuir monolayers of lipids as cell membrane models, and 1,4-Naphthoquinone, which is a potent inhibitor of human cancer cell growth and angiogenesis, was investigated. The drug was incorporated in monolayers of zwitterionic lipids such as DPPC (dipalmitoyl phosphatidyl choline), and negative ones, such as DPPS (dipalmitoyl phosphatidyl choline). Surface pressure-area isotherms showed that the drug induces to a condensation of the monolayer, influences the first-order transition of the lipid from liquid-expanded to liquid-condensed phases, and alters the visco-elastic properties of the monolayers. Also, Polarization Modulation Infrared Absorption-Reflection Spectroscopy (PM-IRRAS) indicated that the drug acts in a first moment in the polar heads of the phospholipids, which causes further distortion of the alkyl chains of the phospholipids. These results are important not only because brings information on drug-membrane interactions at the molecular level, but also because envisage the enhancement of the use of antioneoplastic drugs in cancer treatment.

1275-Pos Board B167
Lipid Membrane Phase Dynamics
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We study lipid phase behavior using giant unilamellar vesicles to model cell membrane dynamics. In our system, we investigate the effects of cross-linking in the head groups position via biotinylated lipids, avidin, and its analogues. Cross-linking is the linking of two molecules (biotinylated lipids) via a crosslinking agent (avidin). Vesicles allow us to isolate the lipid rearrangement due to cross-linking, a common activity on cell surfaces. By comparing specific binding strength of the coupling and self-adhesion, we study the role that cross-linking plays in membrane behavior. Using anti-avidin we attempt to induce aggregation of the membrane bound protein, producing micron size phase domains from initial one-phase vesicles. Confocal microscopy enables us to image this change in the membrane dynamics. Using phase specific dyes, we probe phase segregation on the nanometer scale from the addition of a cross-linker to the system. Förster Resonance Energy Transfer (FRET) enables us to detect clustering on the submicron (1-10 nm) scale, beyond the limits of conventional microscopy. Both techniques allow us to quantify the phase behavior due presence of the cross-linking agent. Using FRET we detect lipid rearrangement associated with the transition from one-phase vesicles to two-phase vesicles using two different fluorescent dyes, a donor and acceptor. From judicious choice of donor and acceptor dyes, we detect the changes in fluorescence acceptor signal as a function of clustering. We are pursuing lifetime studies to complement our current FRET analyses. From this simple cross-linking system, we model membrane responses to protein complex formation and oligomerization.

1276-Pos Board B168
Meta-Cresol Affects Lipid Raft Organization in Membrane-Model Systems and Increases Membrane Leakage in Neural Cells
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m-cresol is an excipient stabilizer used in numerous pharmaceutical formulations, including injectable insulin and vaccines. Therefore, we studied the effects of *m*-cresol in a range of concentrations from 10nM to 3mM on membrane model systems mimicking lipid-rafts and living neural-cells.
First, the intrinsic fluorescence of *m*-cresol was studied. Both its fluorescence lifetime and anisotropy increased in the presence of liposomes, indicating a decreased mobility of the molecule. This interaction was dependent on membrane lipid composition. To elucidate this process, liposomes were labeled with several membrane probes spanning a range of in-depth locations and with preference for distinct lipid domains. For the probes located in the bilayer core (DPH and trans-parinaric acid), no effect was detected even for an *m*-cresol concentration of 300M, whereas for the more superficial NBD-DOPE and NBD-DPPE, >30M *m*-cresol induced a significant fluorescence lifetime decrease.
Atomic force microscopy experiments were performed on ternary supported lipid bilayers containing raft-like liquid ordered domains (Lo). Indeed, it was observed that upon addition of *m*-cresol in the M range, a reduction of the Lo occurs without changing their thickness. For higher *m*-cresol concentrations, raft-like domains are not detected at all.
Whole-cell voltage-clamp recordings from pyramidal-neurons isolated from the CA1 region of rat hippocampus (p21-p29) and from N1E-115 neuroblastoma cells were also performed. *m*-Cresol was applied during constant superfusion and the following parameters were monitored: series-resistance, whole-cell capacitance, holding-current (Vm=-70 mV), and another read-out for the leak-current. Results show that only the leak current was altered by *m*-cresol (>100 M).
As a whole, we show that *m*-cresol interacts with the membrane, affecting lipid raft organization, with functional implications on neural-cell integrity.
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1277-Pos Board B169
Two- Dimensional Macroscopic Protein Domains Induced by the Interplay between Lipid- Protein and Protein- Protein Interactions
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It has been suggested that lipids and proteins are not homogeneously distributed in cell membranes; they can segregate into dynamic micro/ nanodomains, serving as centers for signal transduction, membrane trafficking, and cytoskeletal organization. Here we ask the question whether two- dimensional protein

domains can be created by the interactions between membrane- anchored multivalent proteins. Utilizing the binding pairs, SH3 (SRC homology 3) and PRM (proline-rich motif), which were recently reported to form phase-separated micro-droplets in solution [Li et al., 2012, Nature, 483, 336-340], with histidine tags allowing efficient binding to lipid membranes containing nitrilotriacetic acid (NTA) lipids, we demonstrated that macroscopic protein domains appeared in both giant unilamellar vesicles (GUVs) and Langmuir monolayers. In GUVs, these domains remained circular over a large range of temperatures and protein concentration ratios. In Langmuir monolayers, domains showed reversible transitions from circular shapes to fractal ones depending on surface pressures. Overall, we have demonstrated that the interplay between lipid- protein and protein- protein interactions can induce phase separation of proteins on model membranes.

1278-Pos Board B170

Inhalation Anesthetics Change the Domain Structure of Model Ternary Lipid Raft Membranes

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The mechanism of action for volatile anesthetics remains obscure despite clinical use for over 150 years. No single ion channel or protein receptor appears necessary and sufficient to account for anesthetic action, and the physical effects of volatile anesthetics on homogeneous model membranes appear too small to produce anesthesia. We recently reported that halothane changes the domain structure of a binary lipid mixture[1], increasing the ratio of disordered phase to ordered phase.

We have now studied two ternary model lipid raft mixtures with X-ray diffraction: Dioleoylphosphatidylcholine (DOPC)/dipalmitylphosphatidylcholine (DPPC)/cholesterol, and Dioleoylphosphatidylcholine (DOPC)/sphingomyelin (porcine)/cholesterol. Multi-layers were prepared upon glass slides, hydrated overnight at 98% relative humidity, and maintained at 27.0 $\pm$ 0.1 C on a Peltier-controlled stage in a sealed X-ray chamber. Volatile anesthetics were introduced as solutions in hexadecane. For both raft mixtures, two series of lamellar diffraction peaks are observed, with d-spacings differing by about 10%. These correspond to the liquid ordered phase and the liquid disordered phase. The relative intensities of diffraction for these phases change with increasing temperature and anesthetic concentration, both favoring the liquid disordered phase. A variety of different volatile anesthetics–halothane, isoflurane, chloroform, and hexane_all produce significant increases in the ratios of liquid disordered to liquid ordered lipid phases in these mixtures. These shifts occur at clinically relevant concentrations and are reversible upon withdrawal of anesthetic. There were no consistent effects of the anesthetics on the d-spacings of the lipid layers.

These findings suggest that some effects of volatile anesthetics may be mediated through physical changes in membrane domain structures that interact with membrane proteins.

1. Weinrich, M., Nanda, H., et al., Halothane changes the domain structure of a binary lipid membrane. Langmuir, 2012. 28(10): p. 4723-8.

1279-Pos Board B171

The Effect of Photosensitization on the Physical Properties of a Biological Membrane

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Depolarization of the Nernst electric potential on cells' membranes has been observed in cellular photosensitization, but it was not established whether lipid oxidation is a relevant factor leading to abolishing the resting potential of cells' membranes and to their death. In this work, we studied the effect of liposomes' lipid composition on the kinetics of membrane electric depolarization that is induced by photosensitization. We have studied this effect by two methods: 1. measuring the dissipation of K^+-diffusion electric potential that was generated across the membranes by employing an electrochromic voltage-sensitive spectroscopic probe that possesses a high fluorescence signal response to the potential. 2. Measuring the permeation kinetics of large fluorescent dye molecules, which are known to exhibit self-quenching of their fluorescence at high concentration, through the membranes by observing the increase of the fluorescence as their permeability through the membrane increases. We found a correlation between the structure and degree of unsaturation of lipids and the leakage of the membrane, following photosensitization. As the extent of non-conjugated unsaturation of the lipids is increased from 1 to 6 double bonds, the kinetics of depolarization become faster. When liposomes are composed of a lipid mixture similar to that of natural membranes and photosensitization is being carried out under usual photodynamic therapy (PDT) conditions, photodamage to the lipids is not likely to cause enhanced permeability of ions through the membrane, which would have been a mechanism that leads to cell death.

1280-Pos Board B172

Impact of Oxidized Phospholipids on Membrane Organization

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Lipids have often been seen as basic structural membrane subunits with proteins doing the actual work. This view has changed in recent years where it has been shown that lipids are also directly involved in numerous physiological processes and are often required for specific membrane protein functions. However, how a membrane and its function becomes modified under intracellular oxidative conditions, which e.g. trigger mitochondria-mediated apoptotic cell death, is still not really known. Oxidative stress can generate oxidized phospholipids (OxPls), which have a great impact on mitochondrial membrane integrity. Therefore, we studied the impact of OxPls on DMPC based bilayers membranes by differential scanning calorimetry (DSC) and solid state nuclear magnetic resonance (NMR) spectroscopy. Incorporation of OxPls with functional groups (carboxyl or aldehyde) at their truncated sn-2-chain ends generated information about the effect which OxPl species exert on the basic structural and physico-chemical properties of DMPC bilayers. DSC experiments revealed significant changes in the thermotropic phase behavior of these vesicles in the presence of OxPls as a function of their concentration. In addition, solid state 31P NMR provided molecular information of the behavior of the DMPC headgroups when OxPls were present. In addition changes could also be monitored during temperature induced phase transitions, where OxPls induced a very complex phase behavior. Between 293 K (onset of Lα-phase) and 298 K two overlapping NMR subspectra occured which indicated the co-existence of two liquid-crystalline lamellar phases. Most likely one phase reflected an OxPls poor domain and the other an OxPl-rich domain. In summary, the presence of OxPls seems to alter the mitochondrial membrane organization, which has serious implication for the role of this membrane and its Bcl-2 proteins involved in mitochondrial apoptosis.

1281-Pos Board B173

The Location of Vitamin E in Model Membranes and its Effect on Oxidation

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There are no proven health benefits to supplementing with Vitamin E, so why do we require it for healthy living? The whole notion that vitamin E is an in-vivo antioxidant is now being seriously questioned. We believe the debate in literature is due to much of the existing data being collected using techniques which require the presences of non-biological and invasive probes, and often in the wrong model systems. Using neutron diffraction, supported by solid state 2H NMR, we have correlated vitamin E's location in model membranes with its antioxidant activity. Experiments were conducted using phosphatidylcholine (PC) bilayers whose fatty acid chains varied in their degree of unsaturation. PC bilayers made up of mixed acyl chains (i.e., saturated and unsaturated) and different headgroup moieties were also studied. UV/Vis spectroscopy studies were conducted to examine vitamin E's oxidation at its various locations within the different model membranes. Both water soluble and lipid soluble initiators were used to start the oxidation process.

We observe vitamin E up-right in all lipids examined, with its overall height in the bilayer lipid dependant. Interestingly we observe vitamin E's hydroxyl in the headgroup region of the bilayer for both the fully saturated and poly unsaturated lipids. Vitamin E was most effective at intercepting water borne oxidants